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PRINCIPAL INVESTIGATOR: Hua Wang
Amy Moser

CONTRACTING ORGANIZATION: University of Wisconsin-Madison
Madison, WI 53705

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Hua Wang, Amy Moser

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14. ABSTRACT
Breast cancer develops through multiple steps which are rigorously controlled by genetic factors. It is essential to identify and characterize genes controlling the development of breast cancer to better understand factors affecting tumor susceptibility and contribute to better diagnosis and treatment. We are using a well characterized mouse model, ApcMin/+ mice, to identify genes important for breast cancer progression and development. As the first step to identify modifier gene, we backcrossed B6-ApcMin/+ to the resistant strain FVB. Four novel modifier loci have been mapped to influence different aspects of mammary tumor development in ApcMin/+ mice. Analysis of tumor development in a backcross of (FVBB6 ApcMin/+) x B6 ApcMin/+ mice has identified a modifier on chromosome 9 that significantly affects tumor multiplicity, and a modifier on chromosome 4 that significantly affects tumor latency and affects tumor number with suggestive significance. This modifier was also identified in a backcross involving 129X1/SvJ and B6 ApcMin/+ mice. A modifier on chromosome 18 specifically affects tumor latency but not tumor number. Kaplan-Meier analysis suggests there is at least an additive interaction affecting tumor latency between the loci on chromosomes 4 and 18. I also identified a modifier locus on chromosome 6 that interacts with the loci on chromosome 4 and chromosome 9 to affect tumor number. To further identify genes underlying these modifier loci, I genereated and tested congenic mice on chromosome 4 and 9. Preliminary analysis provide evidence for the modifier on chromosome 4.

15. SUBJECT TERMS
Cancer genetics, Apc, mammary modifier, mammary hyperplasia, mammary tumors

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Introduction

Breast cancer develops through multiple steps which are rigorously controlled by genetic factors. It is essential to identify and characterize genes controlling the development of breast cancer to better understand factors affecting tumor susceptibility and contribute to better diagnosis and treatment. We are using a well characterized mouse model, ApcMin/+ mice, to identify genes important for breast cancer progression and development. ApcMin/+ mice are predisposed to both intestinal and mammary tumors due to a mutation on the tumor suppressor gene Apc. Genetic background affects the susceptibility to mammary tumor development in ApcMin/+ mice. Upon exposure to the alkylating agent ENU (Ethylnitrosourea), more than 90% of B6-ApcMin/+ female mice develop mammary squamous cell carcinomas, with an average 3.3 tumors per mouse and a mean latency of about 56 days. FVBB6 F1 ApcMin/+ mice are resistant to mammary tumors but susceptible to focal alveolar hyperplasias under the same treatment . This suggests that FVB carries alleles at modifier loci of ApcMin that act dominantly to affect the progression of hyperplasias to tumors.

As the first step to identify modifier gene, we backcrossed B6-ApcMin/+ to the resistant strain FVB. Four novel modifier loci have been mapped to influence different aspects of mammary tumor development in ApcMin/+ mice. Analysis of tumor development in a backcross of (FVBB6 ApcMin/+) x B6 ApcMin/+ mice has identified a modifier on chromosome 9 that significantly affects tumor multiplicity, and a modifier on chromosome 4 that significantly affects tumor latency and affects tumor number with suggestive significance. This modifier was also identified in a backcross involving 129X1/SvJ and B6 ApcMin/+ mice. A modifier on chromosome 18 specifically affects tumor latency but not tumor number. Kaplan-Meier analysis suggests there is at least an additive interaction affecting tumor latency between the loci on chromosomes 4 and 18. I also identified a modifier locus on chromosome 6 that interacts with the loci on chromosome 4 and chromosome 9 to affect tumor number. These results suggest that multiple genetic loci control different aspects of mammary tumor development. None of these modifiers is associated with intestinal tumor susceptibility which indicates that these modifiers act on tumor development in a tissue –specific manner.

To better understand the progression and development of mammary tumors, I plan to

1 Generate and test the modifier congenic mice.

2 Characterize the mechanism of modifier.

3 Identify genes regulated in expression during development of mammary tumors through microarray analysis.

Body

I have listed below each of the tasks outlines in the Statement of Work form the original grant proposal. After each task, the progress is described.

Task 1: Test B6-Mmom1 and B6-Mmom3 consomic substitution strain

- Production 20 CSS mice for chromosome 4 and 9 and treat with ENU Month1-3

Progress: This task is complete now. A total of 43 N6 B6-Chromosome 9 congenic Min/+ females were produced. A total of 80 N6-N8 B6-Chromosome 4 congenic Min/+ females were produced.

- Palpate mice weekly and dissect the CSS mice Month 1-4

Progress: This task is complete now. Both N6 chromosome 4 and N6 chromosome 9 congenic mice were treated with ENU, palpated weekly and sacrificed.

- Collect the data about hyperplasia and tumor and do statistic analysis Month 1-4

Progress: Preliminary analysis of chromosome 4 and chromosome 9 congenic mice is complete and this result provides the basis for future studies.

Task 2: Produce the Congenic mice at N10 and analyze congenic mice

- Keep backcrossing the male carrier with B6 till N10 generation Month 1-6

Progress: This task is complete now. N10 B6-chromosome 4 congenic mice are available now.

- Break the chromosome into 4 smaller piece regions Month 6-8

Progress: This task is complete now. 4 lines with overlapping regions have been produced. More recombinant mice between *D4Mit193* and *D4Mit82* will be produced depending the results of analysis of

- Cross the congenic mice with B6 Min/+ Produce 25 female mice for each congenic mice and treat with ENU Month 6-12.

Progress: This work is still in the progress. I expect to produce 35 females for each line and this has taken longer than expected. At this point, I have produced 75 females with different recombinant regions.

- Palpate the mice weekly and dissect the 100 mice Month 8-14
- Collect and process the mammary glands and tumors Month 8-14
- Evaluate the stained mammary glands and process hyperplasia 10-16
- Statistic analysis of congenic mice Month 14-16

Progress: These tasks are still in progress

Task 3: Map the modifier genes to 2 cM region
Task 4: Molecular characterization of congenic mice
Task 5: Microarray analysis of congenic mice
Task 6 Haplotype construction and SNP analysis

Progress: Task 3-6 are still pending because I am waiting for the result from task 2 to make decision about the future work.

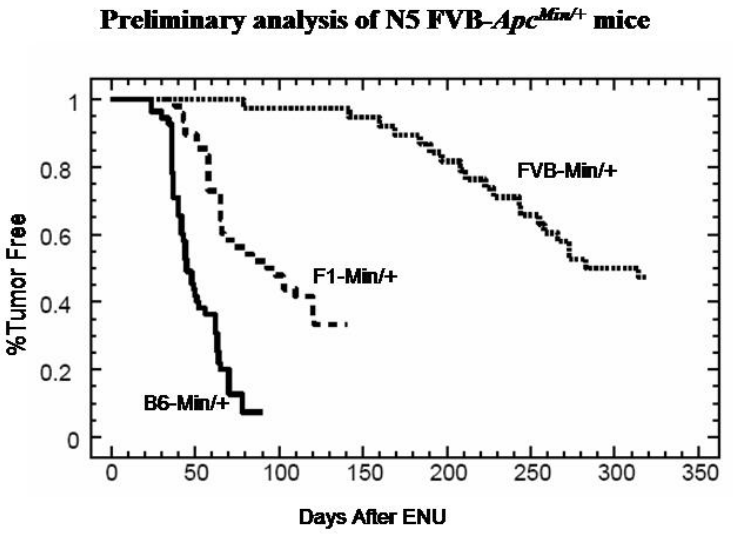
New task: Characterization of mammary tumors from FVB-Min/+ Mice

Progress: At the same time as I generated the mice congenic for the FVB region on chromosome 4 and chromosome 9 on the B6 background, I also generated FVB-*Min*/+ mice so as to be able to compare the phenotype with both parental types. A total of 77 N5 FVB- *Min*/+ female mice were

treated with ENU and sacrificed when they developed tumors or when they reached 300 days after ENU. The FVB-Min/+ female mice developed significantly fewer mammary tumors with longer tumor latency than B6-Min/+ mice. The most interesting phenotype we observed is the mammary tumor type in FVB-Min/+ females. While all mammary tumors from B6-Min/+ mice are squamous cell carcinomas, all mammary tumors from FVB-Min/+ mice are either adenocarcinomas or adenosquamous carcinomas. Now I am focusing on characterizing the molecular differences between adenocarcinomas and squamous cell carcinomas, particularly the effect of Wnt pathway components on mammary tumor development. We will then compare the tumors arising in the
congenic mice from those of the B6 and FVB mice.

Table Single marker analysis of B6-Chr4 N6-N8 congenic mice with tumor number

Marker Name	Position	*Mmom2BB* (No.)	*Mmom2BF* (No.)	P-value
D4Mit193	32.45Mb (7.5cM)	3.62±2.1 (37)	2.61±1.7 (28)	0.04*
D4SNP48.3	48.29Mb	3.81±2.1 (36)	2.48±1.6 (27)	0.00944*
D4SNP54.9	54.92Mb	3.72±2.2 (36)	2.59±1.6 (27)	0.0282*
D4Mit82	63.95Mb (32.5*cM*)	3.56±2.2(39)	2.62±1.6 (26)	0.0756
D4SNP65.0	65.04Mb	3.55±2.2 (38)	2.73±1.6 (26)	0.15
D4Mit45	86.55Mb (42.5cM)	3.38±2.1 (39)	2.59±2.0 (26)	0.306
D4Mit12	123.81Mb *(*57.5*cM)*	3±1.8 (40)	3.48±2.2 (25)	0.552
D4Mit13	141.91Mb((71*cM*)	3.16±1.9 (45)	3±2.3(14)	0.685



Summary:
Chromosome 4 congenic mice: The preliminary analysis of the chromosome 4 congenic mice provides support for a modifier affecting tumor number that maps between *D4Mit193* and *D4Mit82* (Table 1). Thus, we will concentrate on identifying recombinants in that region for further analyses.

KEY RESEARCH ACCOMPLISHMENTS

1 Test both chromosome 4 and chromosome 9 congenic mice and provide strong evidence for the existence of the modifier on chromosome 4.

2 Generate N10 chromosome congenic mice.

3 Find genetic background can change mammary tumor types.

REPORTABLE OUTCOMES:

Hua W, Teske D, Moser A Identification of Novel Modifier Loci of *Apc*^{Min} Affecting the Development of Mammary Tumors (ready to submit)

Hua W, Sullivan, R, Moser A, Genetic background can affect mammary tumor types. (In preparation)

Meetings and Poster: Genetic Dissection of mammary tumor development in *Apc*$^{Min/+}$mice. 20th Mammalian Genome conference, Charleston, SC Nov 12-16.

Conclusions: The tasks in statement of work are complete on time. I have finished testing modifier congenic mice on chromosome 4 and 9. To increase the statistical power, I produced 3 times more congenic mice than planned (120 vs 40). Preliminary analysis confirmed that there is a modifier on chromosome 4 affecting tumor number though the modifier on chromosome 9 fails in statistic analysis. I decided to focus on chromosome 4 modifier and have generate N10 fully congenic mice. Several different lines for chromosome 4 congenic mice were produced and in the middle to test.

I am also working on a new task, which is not listed on the statement of work but of great interest for breast cancer biology. I found the mammary tumors from FVB-Min/+ are adenocarcinomas while mammary tumors from B6-Min/+ are squamous cell carcinomas. I am working to elucidate the mechanism of the tumor fate decision.

REFERENCES: N/A

APPENDICES: N/A

SUPPORTING DATA: N/A